ISTITUTO SIEROTERAPICO MILANESE

Feb.8, 1953.

Dear Joshua,

Much of my time is spent on Congress organization, a very dull task, but I try to keep as active as I can in the laboratory. Some time has elapsed since I got your ~~letter~~ Christmas letter ; thank-you for it, as well as for the offprints of the fundamental Salmonella paper, and the very good Lac paper by Mrs. Lederberg. I am sending under separate cover four copies of offprints -the Heredity paper and the abstract from the Italian microbiology Congress. Tell me if you want more copies. Dealing first with personal business :

1) I have not yet prepared the microfilm of the paragglutination papers but shall do so next week/I hope you were in no hurry/

2) I have sent you under separate cover a complete collection of the Boll.Soc.Int.Microb.Sez.Ital., except years 30-31-32. Publication was stopped in '43. There is no charge for it : we still have a few copies left.

3) You dox not need to thank me for the above. In fact I am already thinking of asking you something on exchange, and exactly, one or two of the U-tubes which you use for filtration experiments. I have a great difficulty in finding glass filters that filter sterile.

4) A young bacteriologist from Rome, Dr.Calef, has started working with me on K-12 genetics. He has an M.D. and is an active and bright young chap (25) with good experience of bacteriology. He would like to spend next academic year in your laboratory, and is looking - has asked Buzzati to get him one- for a scholarship to this aim. Can you accomodate him after October/? I should also be interested about it because I may be able to offer him a job on his return in your laboratory

5) I doubt that I shall be able to come to the States before the Spring of 1954. This summer we are busy with Congresses ; the only opportunity would have been the CSHS, but I have not been invited. I understand Hayes was invited some months ago. Ihope I shall see you at the Genetics Congress, although Congress time is the worst for talking shop. I wonder if you will be able to stay in Europe some time after Sept.the 12th?

6) Hayes has got : 1) an F^r strain ; 2) an Hfr strain. His F- (58-161/S) is very poorly transducible and gives 70% F- prototrophs when crossed to W 677 F+. I should consider it as a weak F^r. His Hfr, obtained spontaneously from an old culture of 58-161/S transduced to F+ seems from his descriptions entirely akin to mine. He asks my Hfr, because his is Sr^Az^r and he cannot test St-resistance of gametes. I would really see no reason to deny it; I should only ask that it be not circulated to other laboratories.

7) Jim Watson has spent here a few days in January. He has a theory to explain segregations - but the theory, which may help to explain some facts, does not fit quantitatively. I understand he is sending you copy of a manuscript about it.

Turning to my work ,

1) F^r: there is probably a locus loosely linked with methionine (but not with B_1 or colicine E-resistance which are linked with methionine). In fact, crossing F^r which is M- to TLB_1-S^r on minSt Met B_1 the M- recombinants are more often F- than F+. F^r recombinants tested sofar (only a dozen) show : normal recombination rate (F^r has low recombination rate and high incidence of recombination of the St-locus in 80%; segregation into F+ and F-, with 50% frequency, when backcrossed to TLB_1-S^rF+, with one exception giving 100% F-. Three such F^r recombinants, including the last, can be transduced though very poorly, to F+. Maybe F^r itself is transducible buta at a very low rate.
When I heard of Hayes's results with his F^r I tested F+ transfer in recombination using the third M-F- strain in existence, i.e. Mrs. Lederberg's strain ; it gave all F+ recombinants when crossed to TLB_1-F+ with one exception. This strain has the same capacity of adsorbing F+ as TLB_1-F-. There is probably a correlation between a) transducibility to F+ ; b) adsorption capacity ; d) % of F+ recombinants (with complications due to segregation) with various F- strains. I shallsw see if I can get some quantitative data about this.

2) Heat or acetone killed F- (and F+) cells can adsorb F+ ~~almost~~ as much as living cells. I am trying to get the F-receptor in a cell-free condition; if successful, it should permit to make a crucial test of the carrier hypothesis by Hayes, which I do not believe, in spite of the Salmonella analogy. This proof could also be carried out with dead cells, but a soluble receptor would be cleaner. Of course, there might always be the escape, for Hayes, that the receptor does not inactivate the F-virus by adsorbing it, but by making its reproduction in the cell impossible : a rather captious objection. However, the experiment has yet to be done.

3) The quantification of the adsorption experiment, and some other work which I have started on the kinetics (for instance, there is a strong dilution effect : less than 10^6 F+ cells/ml will cause almost no infection) are being made possible by the use of the $StAzT_1$ crossing method. It is now quite reproducible. I use Penassay broth with 1% Difco agar in it, and pour plates with 10^{-4} azide, 10^9 T_1, 10^4 micrograms St, and about 10^9 cells from a preincubated mixture. Recombination rates are as follows : TLB_1-S^rF+ x TLB_1-$Az^r$$T_1^r$F-, 4×10^{-7}; F-xF+,

10^{-6} ; F+x F+, 10^{-7} ; F- x F-, 0 or occasionally 2 or 3 colonies out of the 10^9 parental cells plated. Have you started on the kinetics proper? I should very much like to leave you the physical aspects, and consider rather the biological aspects, life stage and conditions of transfer, and production of F+. I will with you more about it.

4) The only coli-line, the F-agent of which seems to be pretty active on F^r x F^r is the Waksman coli. Unfortunately, I am unable to get a stable F+ infection with Waksman F to any K-12 F- line. I have had to work with menage a trois, which is obviously not as clean as one would like. For instance (cultures enclosed), a methionineless or homocystineless coli Waksman (122/33 which I got from Davis, and is probably allelic to M- of 58-161; syntrophy tests not well done, however), plus $M\text{-}S^rF^r$ (original F^r; = strain No.219 of which a culture is enclosed, and corresponds to a St^r mutant from strain No.8), plus a recombinant from F^r x F+, which is $B_1\text{-}St^sM+Xyl\text{-}Mal\text{-}$, on minimal streptomycin B_1. All recombinants are Xyl+Mal+ (with 1% Xyl±Mal±) and a few B_1-. The same menage a trois would be sterile or almost so if the F-donor were an $M\text{-}St^sF+$ of the K-12 line. However, when F_W is acting as recombination-determiner, the pattern of recombination is different from that of F_K (from K-12); it would seem that F_W determines easily the transfer of M+ (occasionally, never that of TL+, very rarely that of St and linked genes. It would seem a sort of "preferential transduction", although the existence of a low proportion of "co-"transduced markers would seem to make this a different case altogether from Salmonella and K-12 (admittedly more similar to the last one; nothing is filterable through Seitz with the F_W system). The difficulty of having stable F_W transduction has led me to stop temporarily this line; however, the finding of other F+ agents permitting F_rxF_r crosses seems rather interesting, and it ~~would be very~~ seems attractive to try on an F^rxF^r your fertile coli-strains other than K-12. The two F^r strains which I ~~have sent~~ am sending ~~seem rather~~ are not ideal, but if the menage a trois with a S^s foreign coli gives higher yield than a cross between the foreign coli and the $M\text{-}S^rF^r$ strain this may be an indication of an effect. Would you be interested to have it tried in your laboratory with other coli strains ? I hope you will . Another question : have you been able to secure stable F transduction from coli Waksman?

5) Your work with Hfr is very interesting. Could you send me H 313, and W-1578 ? Incidentally, how did you get F- in the P-G- ?

Was it obtained with nitrogen mustard?

I am also sending: which is B_1-Lac-, and gives 100% F- on crossing.